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CASE REPORT

Recurrent ovarian cyst and mutation of the Gsα gene in ovarian cyst fluid cells: what is the link with McCune-Albright syndrome?*

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Isolated peripheral precocious puberty due to recurrent ovarian cysts evokes a McCune-Albright syndrome (MAS). This syndrome associates endocrine dysfunction such as precocious puberty, polyostotic fibrous dysplasia, and "café-au-lait" skin lesions. We report the case of a 3-y-old girl who presented with peripheral puberty with extremely elevated oestradiol level, low LH and FSH levels, and an ovarian cyst that quickly resolved. Skeletal X-rays were normal and she had no café-au-lait spots. GnRH analogue treatment was ineffective. A second ovarian cyst appeared and was completely drained under ultrasonographic guidance. Molecular biological analysis performed on fluid cells revealed the Arg201→His mutation of the Gsα gene described in MAS. Percutaneous aspiration of simple ovarian cyst to detect "MAS" mutation is of interest in the diagnosis of recurrent ovarian cyst. □*McCune-Albright syndrome, mutation of Gsα gene, ovarian cyst aspiration, precocious puberty, recurrent ovarian cyst*

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McCune-Albright syndrome (MAS) is a sporadic disease characterized by the triad of "café-au-lait" spots, polyostotic fibrous dysplasia and hyperfunctional endocrinopathies, in particular precocious puberty in girls (1, 2). These endocrinopathies, involving the gonads, thyroid, and pituitary somatotrophs, have an autonomous function and respond to the signal transduction pathway generating cyclic AMP. Mutations of the α-subunit gene of stimulatory guanine nucleotide binding protein, Gsα, have been reported in endocrine adenoma (3, 4). This mutation, corresponding to substitution of either histidine or cysteine for the arginine 201 residue, has been identified in affected tissues and leads to constitutive activation of the Gs proteins that stimulate cAMP formation (5).

Precocious puberty due to recurrent ovarian cysts in the very young girl raises the possibility of MAS. The peripheral origin of this puberty is confirmed by an excess of oestradiol secretion, with a blunted response of gonadotrophin LH and FSH to the gonadotrophin-releasing hormone test (GnRH test), and lack of response to GnRH analogue treatment (6, 7). In this isolated form previously described by Feuillan, the diagnosis of MAS can be difficult because no other sign of the triad is present and we assumed that the underlying defect of MAS was expressed only in the ovary (8). Here, molecular biology can be of great help by revealing the Arg201→His mutation of the Gsα gene in the affected tissue (5).

Case Report

A young girl aged 2 y 8 months presented breast and pilosity development scored Tanner stage II. Her height was 91.5 cm (+0.3 SD), weight 14 kg and bone age 2 y 6 months. Pelvic ultrasonography showed an enlarged uterus measuring 35 × 15 × 8 mm and the volume of the ovaries was 1 cm^3. LH peak was 6.4 mIU/ml and FSH peak 20 mIU/ml after the GnRH test. Oestradiol level (E2) was < 1 ng/dl. These findings were in favour of central precocious puberty but no treatment was started in the absence of bone age advancement.

Five months later, she measured 98 cm (+1 SD), breast enlargement had progressed to B2/3 and bone maturation had accelerated to 4 y. Oestradiol reached high levels (16 ng/dl); in contrast LH and FSH peaks were < 0.5 mIU/ml after the GnRH test. The uterus increased to 46 × 26 × 20 mm, the volume of the left ovary was 4 cm^3 and of the right 10 cm^3, partly due to a cyst measuring 27 × 15 mm which spontaneously resolved 2 weeks later. Given this picture, McCune-Albright syndrome was suggested but there were no other signs, notably no café-au-lait spots nor fibrous dysplasia on skeletal X-ray. Technetium bone scan was not performed. Complete hypophyseal

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investigations with basal and stimulated levels of thyrotrophic, corticotrophic and somatotrophic secretion, and pituitary MRI were normal. Based on the first endocrine results and spontaneous resolution of the cyst, treatment with long-acting GnRH analogue (D-triptorelin, one injection every 28 days) was initiated. However, breast development progressed and vaginal bleeding with recurrent ovarian cyst (15 mm) occurred after 6 months of treatment. D-Triptorelin was discontinued and replaced by cyproterone acetate.

At the age of 4, there were obvious signs of oestrogen excess: Tanner pubertal stage 3, bone age advanced to 7 y, E2 increased to 4 ng/dl, enlarged uterus measuring 58 × 19 × 15 mm with a right ovarian cyst measuring 45 × 34 mm. Tumour markers were negative. We then decided to puncture the ovarian cyst under ultrasound control. Thirty millilitres of transparent, light yellow liquid were withdrawn percutaneously with a fine needle until complete resolution. Fluid analysis found no malignant cells and an elevated E2 level at 100 ng/dl. The Gα subunit gene was studied by molecular biological techniques. DNA sequencing revealed Arg to His mutation at position 201 of the α subunit of the Gs protein gene in the ovarian cells, but not in peripheral blood leukocytes (Fig. 1). The intensity of the mutant allele on autoradiograph was weaker than the normal allele confirming mosaicism. Fifteen days later, the cyst recurred and measured 26 × 13 mm but decreased to 15 × 10 mm 1 month later. Control oestradiol level was still < 1 ng/dl. Treatment with testolactone was proposed but the family did not accept this medication or follow-up.

At 6 y, she was seen by her general practitioner: her breast development was stable (B2 P1) and there were no melanotic skin lesions or recurrent bleeding, but radiological and endocrine investigations were refused by the parents.

Methods

DNA preparation, PCR techniques and sequencing

A pellet of approximately 10^5 cells was obtained by centrifugation (5000 *g*, 5 min) from 15 ml of aspirated ovarian cyst fluid. The cell pellet was resuspended and incubated at 56°C for 1 h in 30 μl of 1 × PCR buffer (50 mM KCl, 10 mM Tris pH 8.3 mM, 2.5 mM $MgCl_2$) containing 0.5% Tween-20 and 1.5 mg proteinase K. The lysate was then incubated at 95°C for 15 min to inactivate the proteinase K. After brief centrifugation the supernatant containing DNA from fluid cells was isolated and stored at −20°C. For PCR amplification, we used 5 μl of the cell lysate.

Enzymatic amplification was performed in a final volume of 100 μl using 200 ng of DNA, 50 ng of each primer, 200 μM of each nucleotide, 2.5 μM MgCl2, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 0.01% gelatine and 0.5 U of Taq DNA polymerase (Promega, France). Exons 8 and 9 and intron 8 of Gsα gene were amplified by the following set of primers: 5′ TGTTTCAGGACCTGCTTCGC 3′ and 5′ ATCCTACCGTTGAAGCACTG 3′. Amplified samples underwent electrophoresis on a 1.2% agarose gel stained with ethidium bromide to verify the product sizes.

Polymerase chain reaction products were purified on 2% low melting agarose gel (NUSIEVE, FMC, USA). Briefly, bands were excised, agarose gel was remelted at 65°C and DNA was obtained after phenol–chloroform extraction and ethanol precipitation. Sequencing was performed directly on purified PCR products using the dideoxynucleotide termination chain procedure (Sequenase kit, Amersham, France). Sequences were repeated on two separate PCR products and with both sense and anti-sense primers.

Discussion

The clinical observation showed the difficulties of management and of diagnosis between precocious puberty and recurrent ovarian cyst in a young girl. Initially, the patient had findings suggestive of central precocious puberty corresponding to the trigger event, and we can suspect the role of cyclic AMP in both release of GnRH and hypersensitivity of hypophyseal gonadotrophins, either causing secondary activation of an unsuspected pre-existing ovarian cyst. The lack of efficacy of GnRH analogue treatment, the progression of pubertal signs and the recurrence of the cyst indicated that puberty was peripheral and suggested a McCune-Albright syndrome (6). The possibility of such isolated ovarian involvement had been predicted by Feuillan, who described the same clinical features in two girls (8). Somatic mutations of the Gsα gene have been identified in other isolated endocrinopathies, namely somatotroph and thyroid adenoma (3, 4). This mutation can be present in other affected tissue such as isolated fibrous dysplasia of bone (9). Other endocrine dysfunctions were carefully checked and were not present in our observation (10). There were no cutaneous lesions or bone dysplasias to confirm this syndrome, but these two physical signs may appear several years later (11). Indeed, a case is reported in the literature of a 27-y-old woman who presented with fibrous dysplasia 24 y after isolated transient premature menstruation and sexual precocity (12). Some authors insist on the value of technetium^{99} bone scan to detect lesions not visible on X-ray; unfortunately, this was not performed in our patient (13). At the time, we could not detect other signs of McCune-Albright syndrome in our very young patient. The discovery of somatic mutations of the Gsα gene brought molecular confirmation of the previous hypothesis concerning the pathophysiology of the endocrine hyperfunction observed in this disorder. Somatic mutations of the α subunit gene early in embryogenesis could result in the mosaic population of normal and mutant-bearing tissues that may underlie the clinical manifestations of this disease (14). In our observation, the novel and unique finding is that in a case of recurrent ovarian cyst we were able to perform cyst aspiration and found an Arg201→His mutation of the Gsα gene in this affected tissue but which was absent in the DNA of

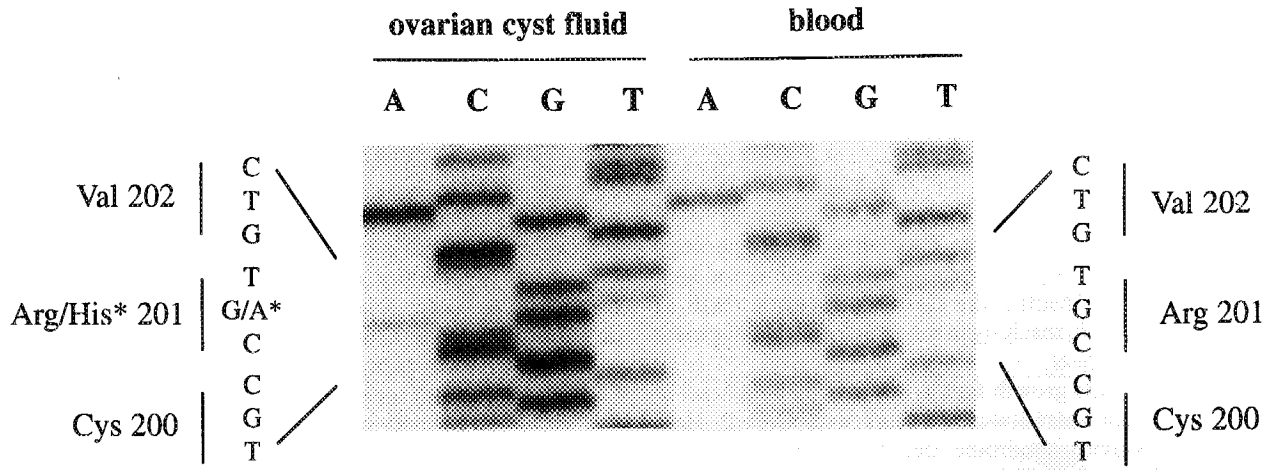

Fig. 1. Autoradiography of the sequencing gel showing the somatic mutation found in DNA extracted from ovarian cyst fluid and the normal sequence in DNA extracted from peripheral blood leucocytes of the patient.

peripheral blood leukocytes. Although we cannot predict at the present time whether other signs of MCA syndrome will occur or not, we believe that the finding of this mutation is of importance for long-term follow-up and for screening other localization.

We wish to emphasize the usefulness of ultrasound-guided aspiration, which is a suitable alternative to surgery in the management of simple ovarian cyst (15). Percutaneous aspiration deserves wider development in order to analyse the Gsα gene in many recurrent secretory ovarian cysts to see if this finding is frequently or unusually associated with this pathology.

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